Rescue potential of supportive embryo culture conditions on bovine embryos derived from metabolically-compromised oocytes

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Metabolic disorders like obesity are linked to subfertility. The upregulated lipolysis leads to elevated saturated (stearic; SA, palmitic; PA) and unsaturated (oleic; OA) fatty acids (FAs), both in serum and in follicular fluid. While their ratio determines the severity of lipotoxicity, exposure to these FAs has a detrimental impact on oocyte quality. Insulin-transferrin-selenium (ITS, as a mitogenic and antioxidative support) is used in *in vitro* culture medium to maximize blastocyst yields. We hypothesize that supportive culture media (containing e.g. ITS) can minimize cellular stress levels and rescue development and quality of embryos derived from metabolically-compromised oocytes. In this study, bovine oocytes were exposed to different ratios of PA, SA and OA; 1) pathophysiological concentrations: 150, 75 and 200 µM respectively (HI COMBI), 2) only high PA: 150, 28 and 21 µM respectively (HI PA), compared to 3) physiological basal concentrations: 23, 28 and 21 µM respectively as a control (BASAL). Presumptive zygotes were cultured in SOF medium with or without ITS. Cleavage rates were recorded 48h post insemination (p.i.) and blastocyst rates at day 7 (D7) and 8 (D8) p.i. (n=905 oocytes, 3 repeats). D8 blastocysts were evaluated for apoptotic cell indexes (n=227) by caspase-3 immunostaining or snap frozen for mRNA expression of genes involved in ER unfolded protein responses (UPR) (*Atf4, Atf6*), oxidative stress (*SOD2, GPx, CAT*) and mitochondrial UPR (*HSPE1, HSPD1*) (n=356). Categorical and numerical data were analysed using binary logistic regression and ANOVA, respectively, and were Bonferroni-corrected for multiple testing. In the absence of ITS during culture, HI PA exposure during maturation significantly reduced cleavage (64.2% *vs.* 78.3%) and D7 blastocyst rates (12.4% *vs.* 24.3%) and tended to reduce D8 blastocyst rates (22.0% *vs.* 32.5% P=0.098) compared to BASAL. No significant differences in development were present in the HI COMBI group. However, surviving blastocysts derived from HI PA and HI COMBI-treated oocytes showed a significant increase in apoptosis. In the presence of ITS, exposure to HI PA or HI COMBI had no significant effect on developmental competence whereas the apoptotic effect was not alleviated by ITS. Within the HI PA group, ITS supplementation rescued embryo cleavage rate (by 15.1%, P<0.05), proportion of ≥4-cell embryos (by 13.9%, P<0.05) and tended to increase D7 blastocyst rate (by 8.2%, P=0.076) compared to the HI PA-treated group cultured without ITS. In the absence of ITS during culture, *HSPD1* expression of D8 blastocysts from PA-treated oocytes was significantly increased compared to BASAL-treated oocytes (P<0.05), an effect that was normalised by ITS. Within the HI PA-treated group, ITS tended to decrease *HSPD1* expression (P=0.069). Other genes were not affected. We conclude that ITS supplementation during embryo culture enhances development and mitochondrial stress responses of embryos derived from metabolically compromised oocytes (HI PA). However, produced embryos still showed higher apoptosis, indicating inferior quality.